THE ACTION OF INVERTASE PREPARATIONS

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Figs. 1 and 2) does ethyl alcohol, in the concentrations used, significantly affect the oxygen uptake of the cells.

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Evidence has been presented by Bacon and Edelman (1), Blanchard and Albon (2), and confirmed by Fischer, Kohtès, and Fellig (3), and White and Secor (4), that several oligosaccharides appear during the reaction of yeast invertase with sucrose, disappearing at the completion of the inversion. Five oligosaccharides, including a tetrasaccharide, two trisaccharides, and probably two disaccharides have been demonstrated chromatographically by White and Secor who have quantitatively determined the monosaccharide composition of four of them (4).

Aronoff (5) has been unable to demonstrate such intermediate compounds and ascribes the results of Bacon and Edelman and those of Blanchard and Albon to contamination of their invertase preparations with a sugar-transferring enzyme. It was implied that the invertase preparation used by him was free of such contamination. The preparation was Wallerstein "Blue Label" invertase scales, a commercial preparation. The paper by Fischer *et al.* (3) confirmed earlier results using highly purified invertase preparations rather than crude or commercial materials.

Aronoff inverted radioactive sucrose spots on paper prior to chromatography by superimposing a 1% solution of invertase, then drying the spots by infrared lamp after intervals of 1–10 min. He presents a radiogram of the irrigated papergram. Observation of the illustration indicates that rather than incomplete action of the enzyme over the entire sucrose spot, what appears to have taken place was essentially complete reaction with only part of the spot of sucrose. As previously pointed out (1–4) the oligosaccharides formed disappear at the end of the reaction.

This interpretation has been confirmed in this laboratory by a repetition of Aronoff's conditions, using enzyme from the same source, but not using radio sucrose. Marked differences in the intensity of the developed sucrose spot resulted when identical sucrose spots were treated with the invertase solution, with one being steamed to inactivate the enzyme before drying while the other was not. The chromatogram of the inversions that had been steamed showed oligosaccharide intermediates; those simply dried by infrared lamp (at not over 35°C. for 1 min.) showed only traces of sucrose, more monosaccharides, and no other oligosaccharides present.

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Steaming, per se, is not required for oligosaccharide formation, but simply is a convenient and rapid means for inactivating the enzyme and stopping the reaction. Preliminary experiments were made using Aronoff's drying technique with a much less active yeast invertase preparation. In this case, oligosaccharide formation was shown on the papergrams dried by infrared at 2 min. The activity of the preparation was so low that the inversion was not complete at the time of irrigation of the paper, hence the persistence of the enzyme-labile (1–4) oligosaccharides.

Reaction of sucrose and invertase for the demonstration of the intermediate sugars is better carried out in solution than on paper. Thus 0.9 ml. of 15% aqueous sucrose was mixed with 0.2 ml. of a 1% solution of Wallerstein "Blue Label"

TABLE I

Oligosaccharides Formed During Sucrose Inversion by Yeast Invertase

Designation[b]	Reducing	Contain ketose[c]	R_s[a]			
			Bacon-Edelman (1)	Fischer *et al.* (3)	White-Secor (4)	This study
Component I	Yes	Yes	0.77	0.75	0.71	0.75
Component II	No	Yes	0.55	0.56	0.41	0.58
Component III	No	Yes	0.43	0.45	0.31	0.48
Component IV	No	Yes			0.12	
Component V	Yes	?			1.0	1.11

[a] Travel of spot ÷ travel of sucrose, calculated from R_f values in papers cited. Variations among laboratories may be due to use of different solvents in chromatography: butanol–acetic acid–water 4:1:5 (1); butanol–pyridine–water 3:1:1.5 (3) and this study; butanol–ethanol–water 10:1:2 (4).

[b] Nomenclature is that of Bacon and Edelman. The designations of Fischer *et al.* originally appeared in the reverse order and are listed here in the order corresponding to that of Bacon and Edelman.

[c] Shown by naphthoresorcinol–phosphoric acid spray reagent.

Invertase Scales.[1] Samples of 0.002 ml. were removed to paper at intervals, and steamed. These were then chromatographed in the conventional manner. Only spots which had been steamed within 2 min. of the addition of the enzyme solution showed the oligosaccharide spots after irrigation. Demonstration by carrying out the reaction on paper was made by development of a papergram of dried 0.002-ml. spots of 15% sucrose followed by these treatments: 0.002 ml. of 1% invertase followed by steaming at 30 sec., or 0.002 ml. of a 0.1% invertase solution followed by steaming at 2 min.

Thus, if transfructosidation is due to a contaminating enzyme, as does not seem likely, the yeast invertase preparation used here, from the same source as that used by Aronoff, contains this contamination.

[1] Mention of trade names does not imply endorsement by the Department of Agriculture.

Bacon and Edelman (1) and Fischer *et al.* (3) described three intermediate oligosaccharides. White and Secor (4) described two additional compounds formed in the action of yeast invertase upon sucrose. We have confirmed the occurrence of four of these five oligosaccharides. Combined data are given Table I.

The fifth component, demonstrated by White and Secor in the leading edge of the sucrose spot, is easily shown by use of a triphenyltetrazolium chloride reagent which does not react with nonreducing sugars.[1] Its proximity to larger amounts of sucrose in the papergram makes it impossible to decide whether it contains a ketose sugar by color reaction on the paper. We have not observed any spot of R_s less than that of component III in this reaction.

The above discussion has been concerned with the fructoinvertase (6) of yeast. Examination of the action of a glucoinvertase (6) preparation from honey on sucrose shows that six oligosaccharides are formed during the reaction and disappear at completion. There is evidence that most if not all of these compounds differ from those listed in Table I since transglucosidation is involved rather than the transfructosidation which is proposed for yeast invertase (7). Further evidence will appear in another communication.

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[1] Fifty mg. of 2,3,5-triphenyltetrazolium chloride is dissolved in a mixture of 10 ml. of 5% NaOH, 30 ml. *n*-butanol and 20 ml. ethanol. After spraying the reagent on the paper, it is developed to the desired contrast by atmospheric steaming. Reducing sugars are the only sugars to react showing intense red color.

[2] This research was carried out under the auspices of the U. S. Atomic Energy Commission.